

#16. 1A

1st Round : screening of mutants (full length Ala-scan)

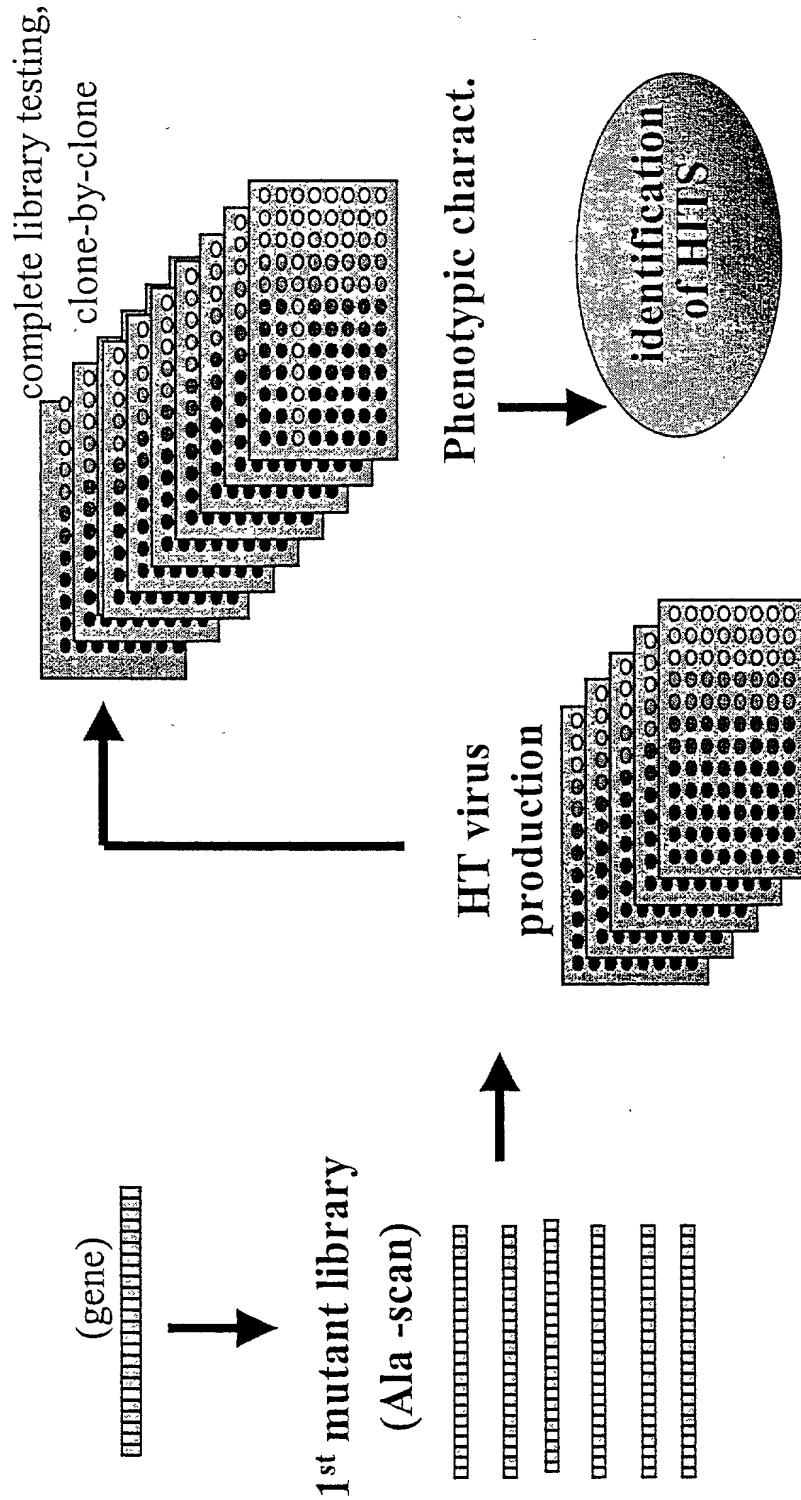
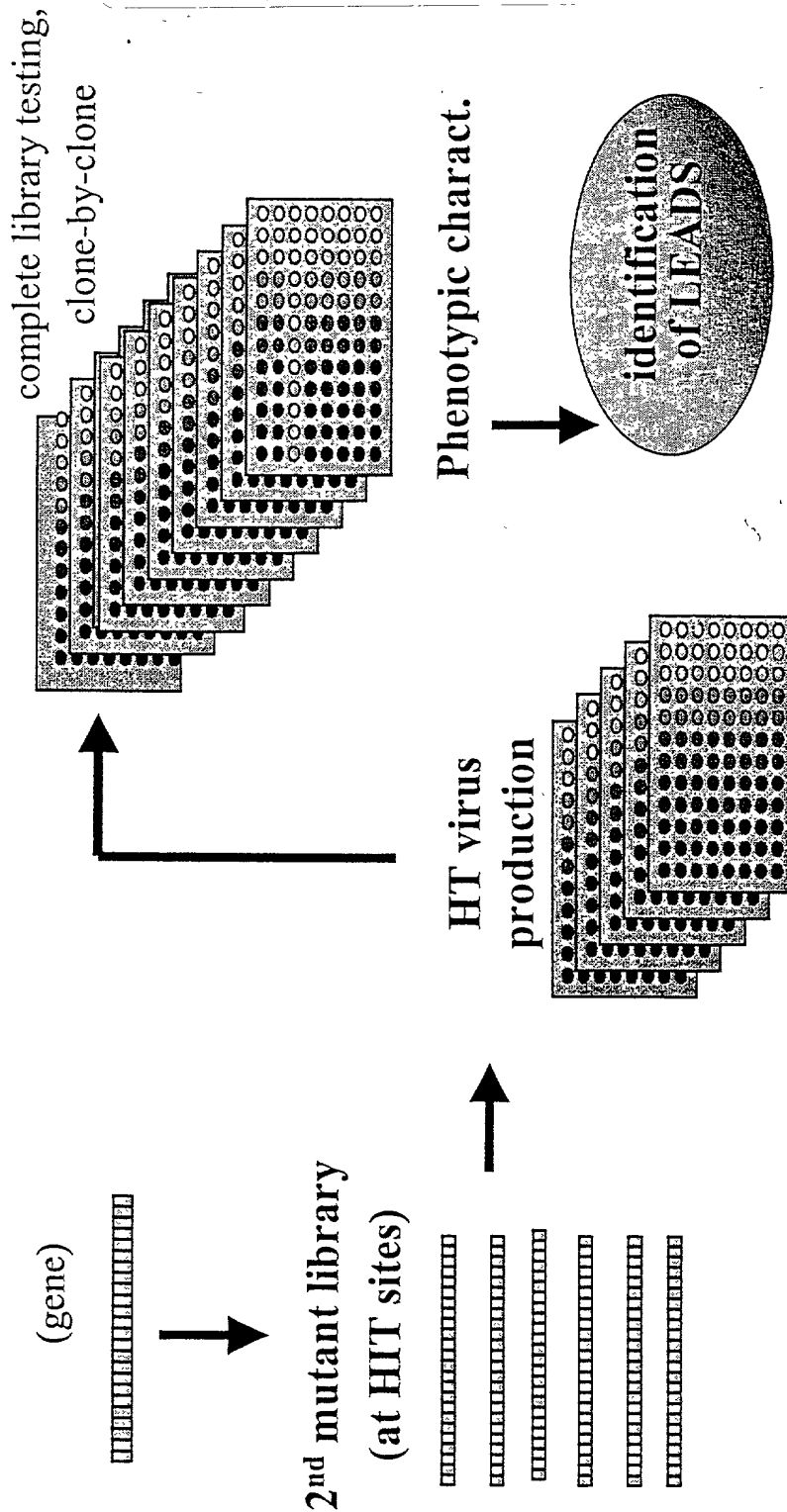


FIG. 1B

2nd Round : screening of mutants at (surrounding) HIT positions



FOI 64222001

FIG. 1C

3rd Round : screening of recombinants between LEADS

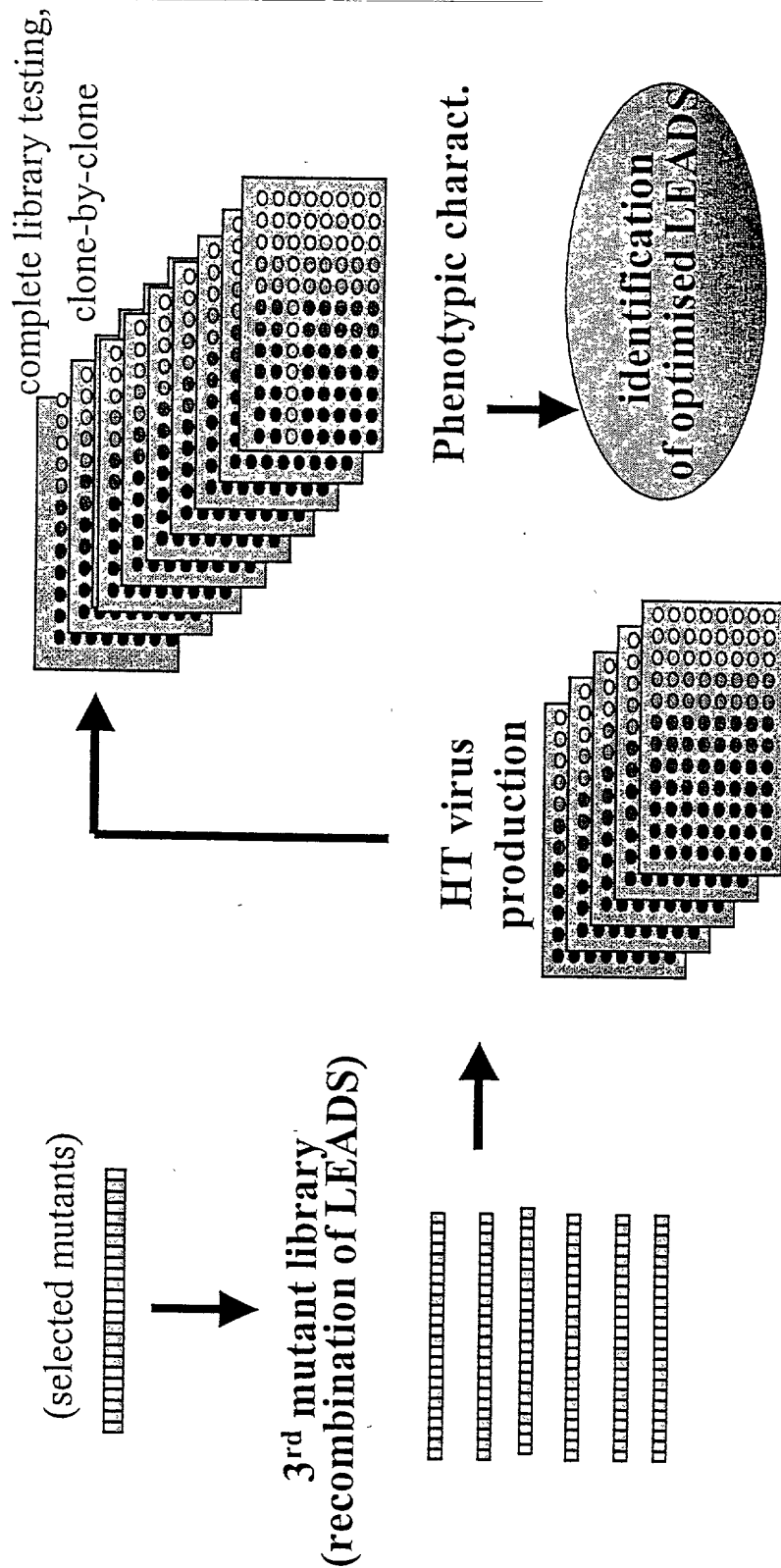
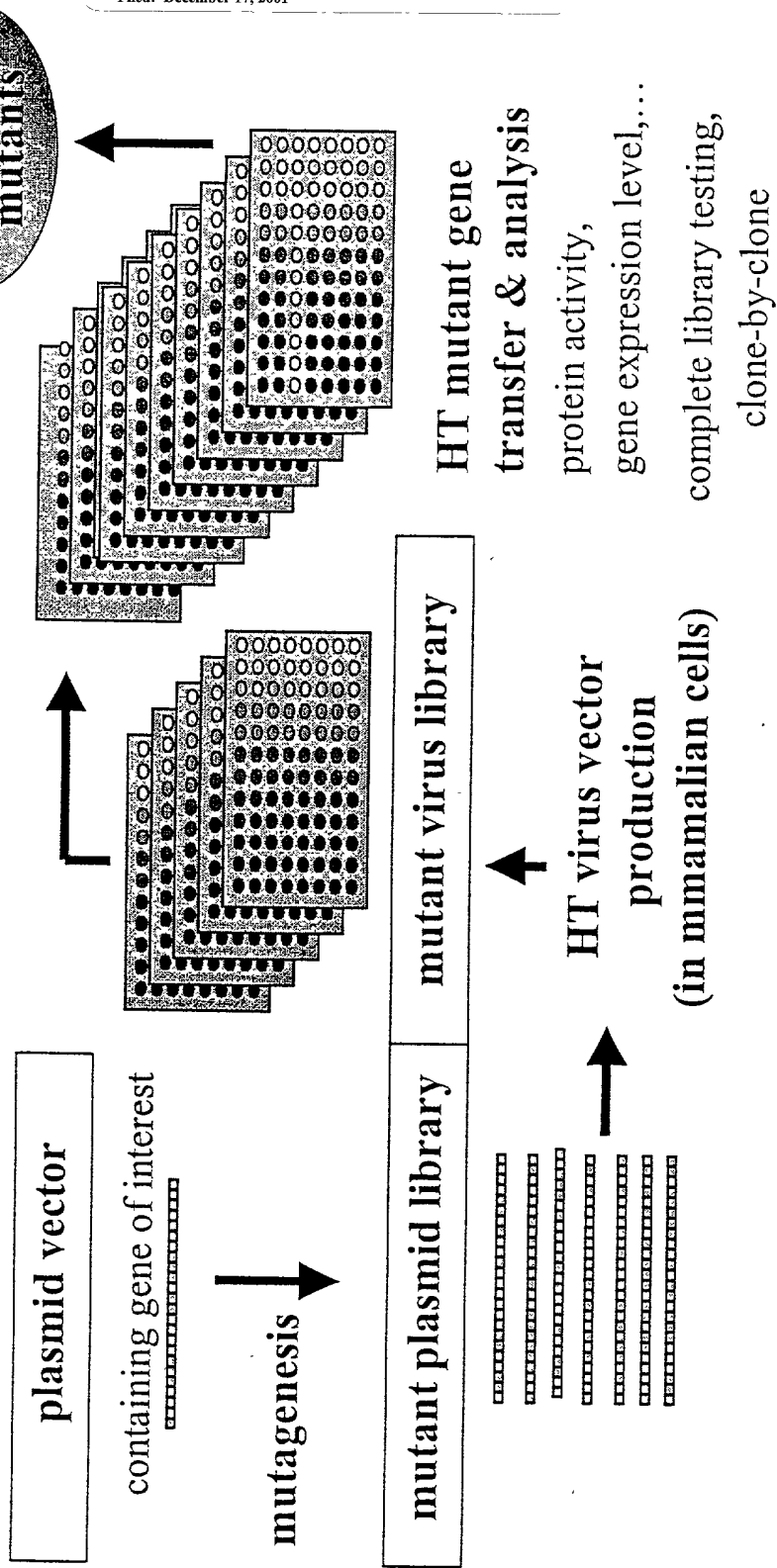


FIG. 1D

Gene optimisation (in mammalian cells)



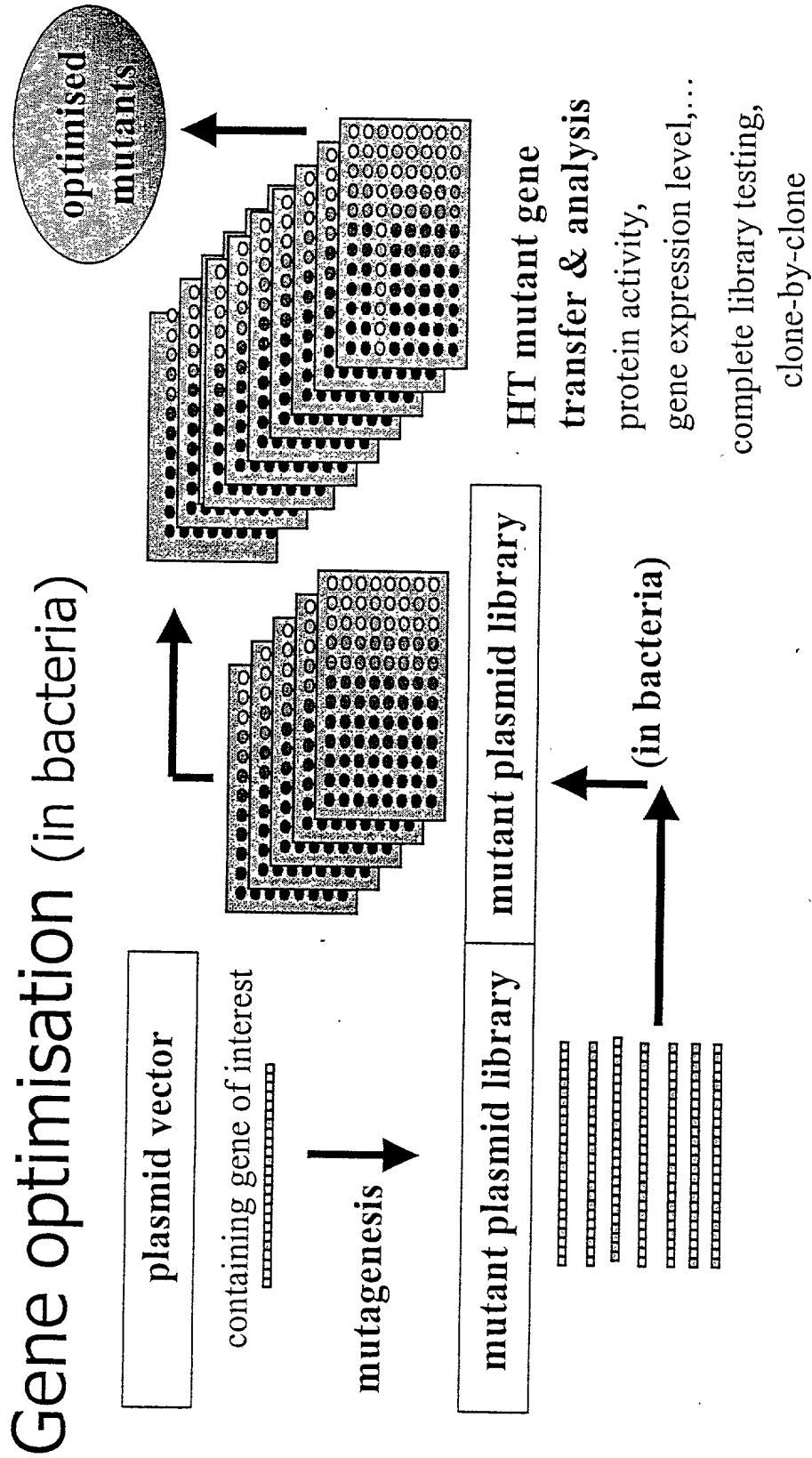


FIG. 2A

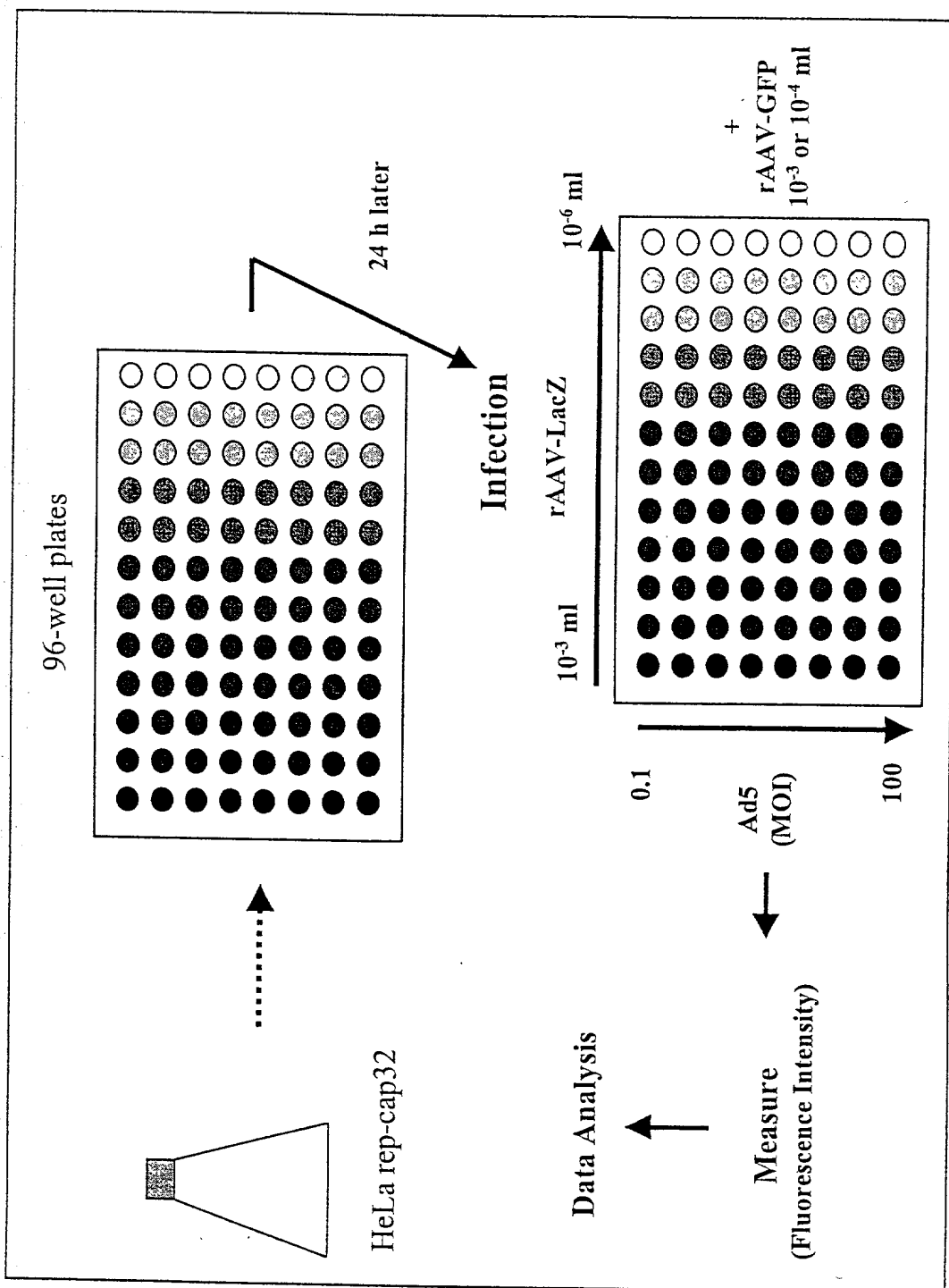


FIG 2B

TREE AAV lacZ 1 μ l+AAV GFP 1 μ l

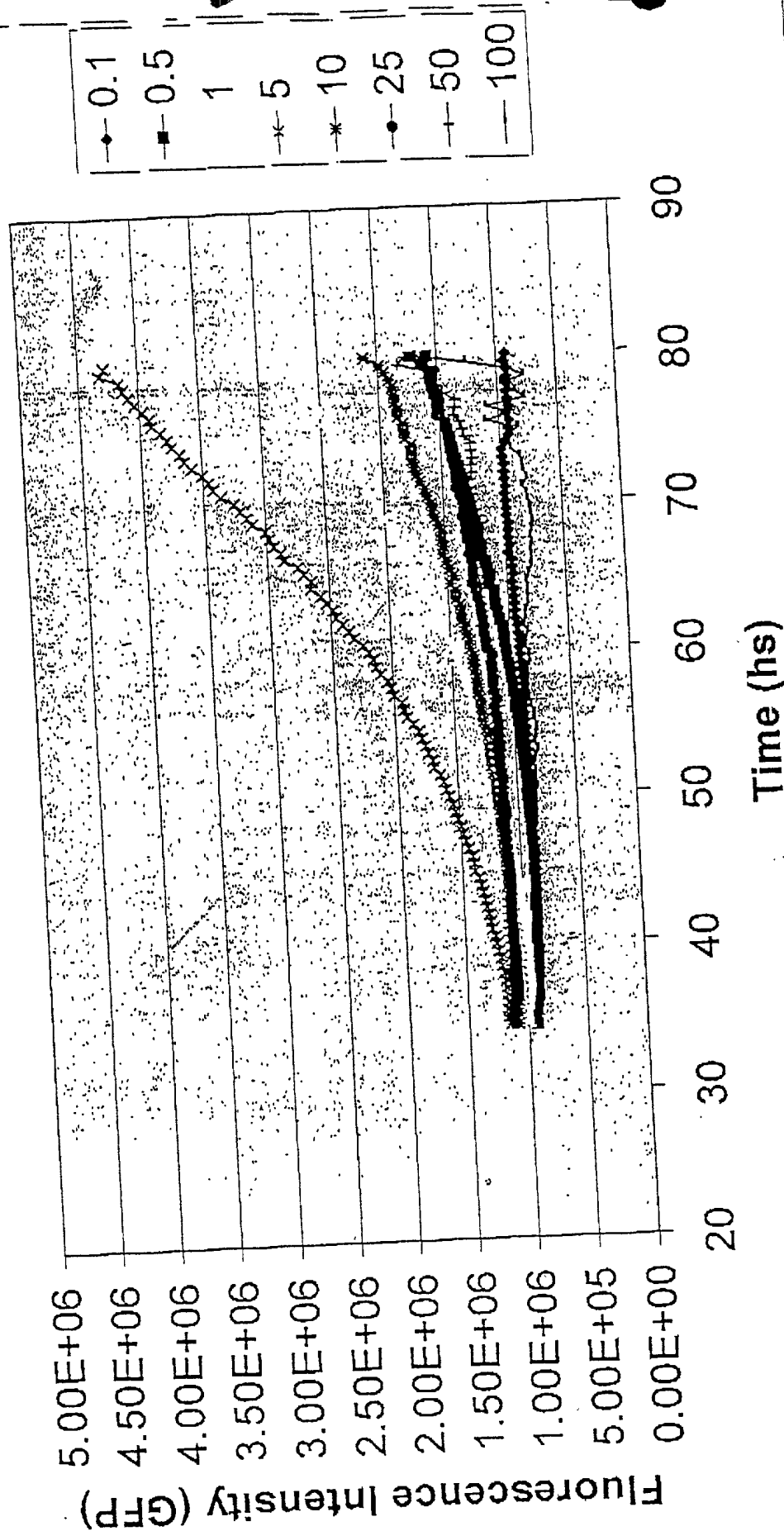


FIG. 2 C

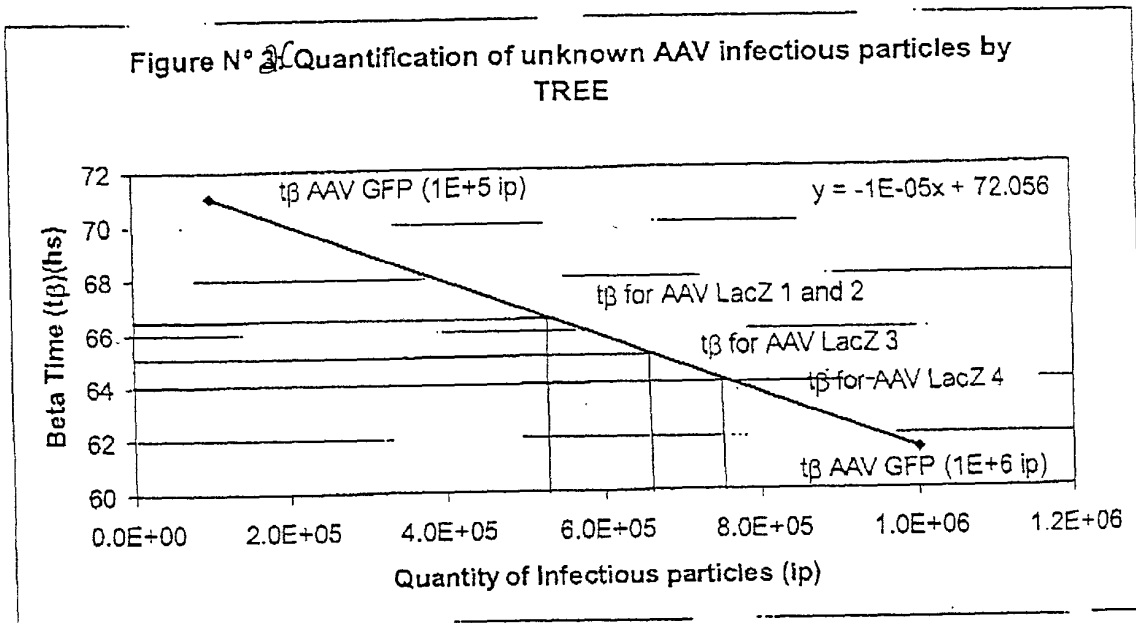


FIGURE 3A

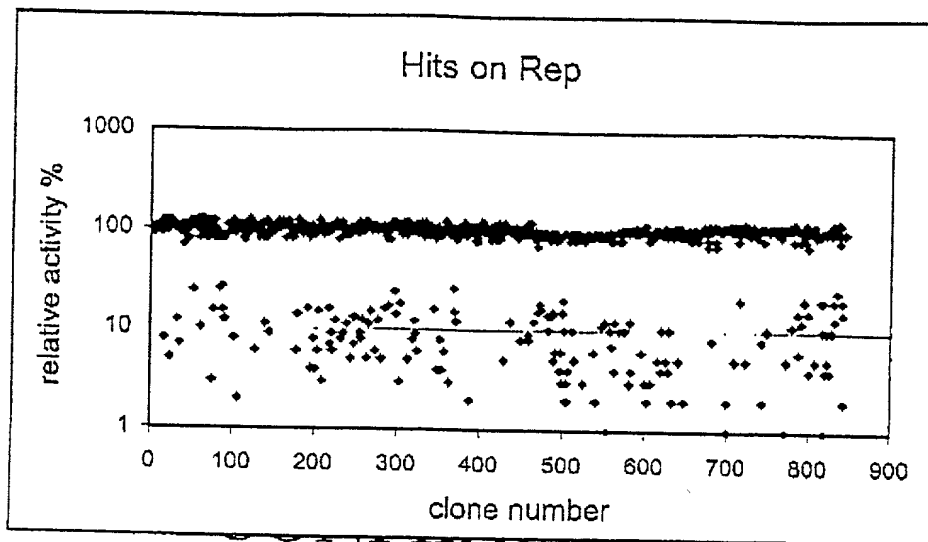


FIGURE 3B

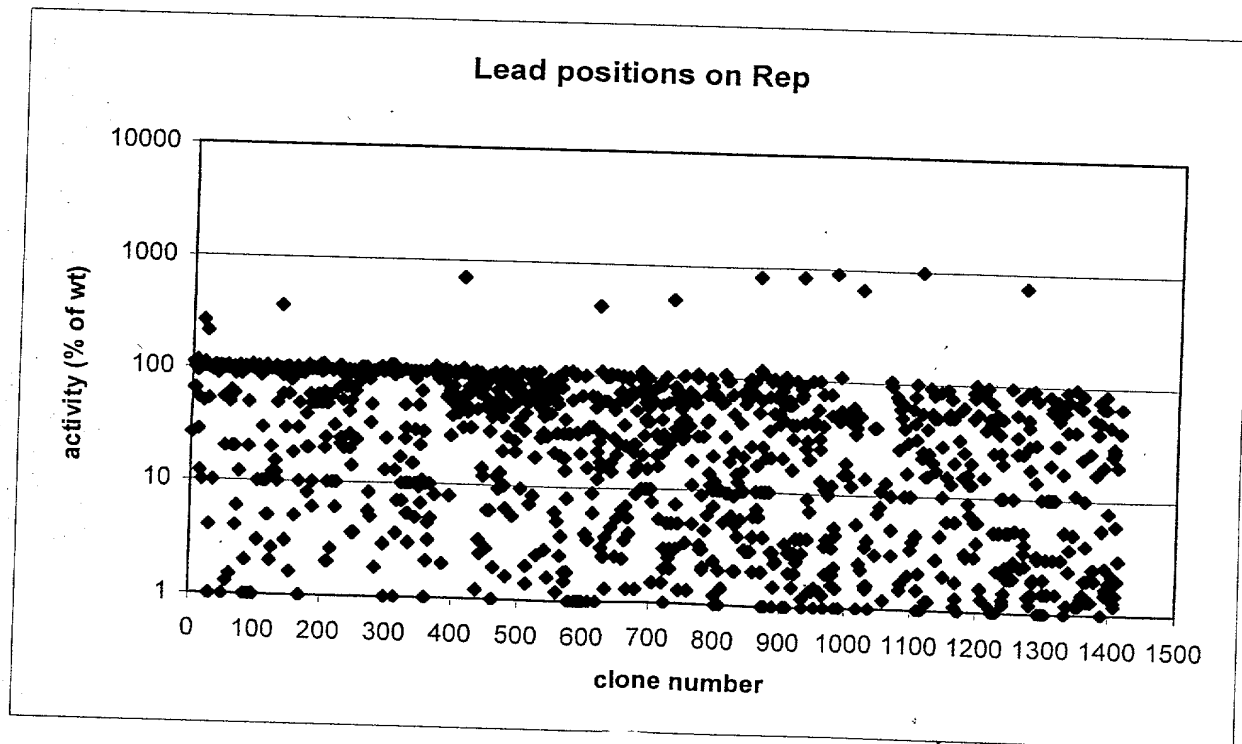


FIGURE 4

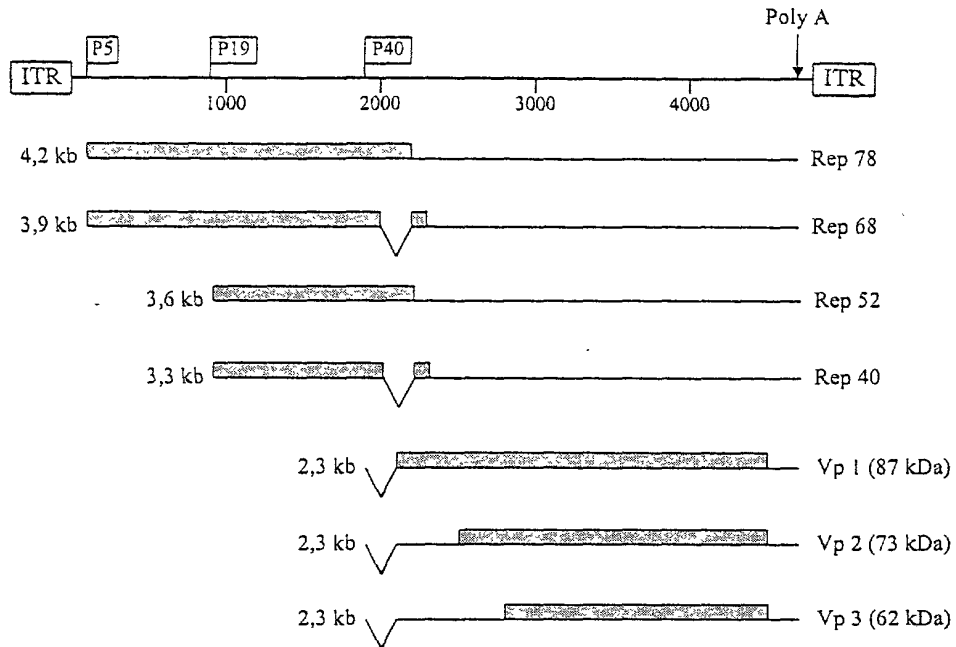


FIGURE 5A

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      10      20      30      40      50      60
1  MPGFYEIVIKVPSDLDEHLPGISDSFVSWVAEKEWELPPDSMDLNLIEQAPLTVAEKLQ 60
2  MPGFYEIVIKVPSDLDEHLPGISDSFVNWVAEKEWELPPDSMDLNLIEQAPLTVAEKLQ 60
3  MPGFYEIVLKVPSDLDEHLPGISNSFVNWVAEKEWELPPDSMDPNLIEQAPLTVAEKLQ 60
4  MPGFYEIVLKVPSDLDEHLPGISNSFVNWVAEKEWELPPDSMDPNLIEQAPLTVAEKLQ 60
5  MPGFYEIVLKVPSDLDEHLPGISDSFVSWVAEKEWELPPDSMDLNLIEQAPLTVAEKLQ 60
6  MPGFYEIVIKVPSDLDEHLPGISDSFVNWVAEKEWELPPDSMDLNLIEQAPLTVAEKLQ 60
7  MATFYEIVIRVPFDVEEHLPGISDSFVDWVTGQIWELPPESDLNLTLVEQPQLTVADRIR 60
C  M**FYE**:*VP*D***HLPGIS+SFV:WV****WELPP*SD**+*L*EQ**LTVA****

      70      80      90      100     110     120
1  RDFLVQWRRVSKAPEALFFVQFEKGESYFHLHLVETTGVKSMVLGRFLSQIRDKLVQTI 120
2  RDFLVQWRRVSKAPEALFFVQFEKGESYFHLHLVETTGVKSMVLGRFLSQIRDKLVQTI 120
3  REFLVEWRRVSKAPEALFFVQFEKGETYFHLHLVLIETIGVKSMVVGGRYVSQIKEKLVTRI 120
4  REFLVEWRRVSKAPEALFFVQFEKGETYFHLHLVLIETIGVKSMVVGGRYVSQIKEKLVTRI 120
5  REFLVEWRRVSKAPEALFFVQFEKGDSYFHLHLVETVGKSMVVGGRYVSQIKEKLVTRI 120
6  RDFLTEWRRVSKAPEALFFVQFEKGESYFHMHLVETTGVKSMVLGRFLSQIREKLIQRI 120
7  RVFLYEWNKFSKQ-ESKFFVQFEKGSEYFHLHVLVETSGISSMVLGRYVSQIRACLKLVV 119
C  R:FL++W***SK**E***FFVQFEKG+:YFH*H:L+ET:G**SMV:GR::SQI::*L*::*

      130     140     150     160     170     180
1  YRGIEPTLPNWFVAVTKTRNGAGGGNKVVDECIYPNYLLPKTQPELQWAWTNMEEYISACL 180
2  YRGIEPTLPNWFVAVTKTRNGAGGGNKVVDECIYPNYLLPKTQPELQWAWTNMEEYISACL 180
3  YRGVEPQLPNWFVAVTKTRNGAGGGNKVVDDCIYPNYLLPKTQPELQWAWTNMDQYLSACL 180
4  YRGVEPQLPNWFVAVTKTRNGAGGGNKVVDDCIYPNYLLPKTQPELQWAWTNMDQYLSACL 180
5  YRGVEPQLPNWFVAVTKTRNGAGGGNKVVDDCIYPNYLLPKTQPELQWAWTNMDQYISACL 180
6  YRGIEPTLPNWFVAVTKTRNGAGGGNKVVDECIYPNYLLPKTQPELQWAWTNMEQYLSACL 180
7  FQGIPEQINDWVAITKVKK--GGANKVVDGYIPAYLLPKVQPELQWAWTNLDEYKLAL 177
C  **G:EP:***W*A*TK*****GG*NKVD:*YIP*YLLPK*QPELQWAWTN*:Y:*A*L

      190     200     210     220     230     240
1  NLAERKRLVAQHLTHVSQTQEQNKENLNPNSDAPVIRSKTSARYMELVGWLVDRGITSEK 240
2  NLAERKRLVAHDLTHVSQTQEQNKENLNPNSDAPVIRSKTSARYMELVGWLVDRGITSEK 240
3  NLAERKRLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWLVDRGITSEK 240
4  NLAERKRLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWLVDRGITSEK 240
5  NLAERKRLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWLVDRGITSEK 240
6  NLTERKRLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWLVDKGITSEK 240
7  NLEERKRLVAQFLAESSQRS-QEAAASQREFSADPVIKSKTSQKYMALVNWLVEHGITSEK 236
C  NL+ERKRLVA*+L***SQ***Q*****S**PVI*SKTS**YM*LV*WLV*+GITSEK

      250     260     270     280     290     300
1  QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVGPPADIKTNRIYR 300
2  QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVGPPADIKTNRIYR 300
3  QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITKNRIYQ 300
4  QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITKNRIYQ 300
5  QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGQNPPEDISSNRIYR 300
6  QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTKTAPDYLVGQNPVEDISSNRIYK 300
7  QWIQENQESYLSFNSTGNSRSQIKAALDNATKIMSLTKSAVDYLVGSSVPEDISKNIWQ 296
C  QWIQE*Q*SY*SFN***NSRSQIKAALDNA:KIM+LTK:A*DYLVG:***+DI::NRI*:

      310     320     330     340     350     360
1  ILELNGYEPAYAGSVFLGWAQKRFGKRNTIWLFGPATGKTNIAEAIHAHVPFYGCVNWT 360
2  ILELNGYDPAYAGSVFLGWAQKRFGKRNTIWLFGPATGKTNIAEAIHAHVPFYGCVNWT 360
3  ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATGKTNIAEAIHAHVPFYGCVNWT 360
4  ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATGKTNIAEAIHAHVPFYGCVNWT 360
5  ILEMNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATGKTNIAEAIHAHVPFYGCVNWT 360
6  ILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATGKTNIAEAIHAHVPFYGCVNWT 360
7  IFEMNGYDPAYAGSILYGWCQRSFNKRNTVWLYGPATGKTNIAEAIHAHVPFYGCVNWT 356
C  I*E+NGY*P:YA:S***GW***:F*KRNT*WL*GPATGKTNIAEAIHAHVPFYGCVNWT
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FOOTNOTES

FIGURE 5B

	370	380	390	400	410	420	
1	NENFPFNDCVDKMWIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIIVTS						420
2	NENFPFNDCVDKMWIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIIVTS						420
3	NENFPFNDCVDKMWIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIEPTPVIIVTS						420
4	NENFPFNDCVDKMWIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIEPTPVIIVTS						420
5	NENFPFNDCVDKMWIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIIVTS						420
6	NENFPFNDCVDKMWIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIIVTS						420
7	NENFPFNDCVDKMLIWWEEGKMTNKVVESAKAILGGSKVRVDQKCKSSVQIDSTPVIIVTS						416
C	NENFPFNDCVDKM*IWWEEGKMT*KVVESAKAILGGSKVRVDQKCKSS*QI+*TPVIIVTS						

	430	440	450	460	470	480	
1	NTNMCVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDGKVTQKEVKEFFRWAQDHVTEV						480
2	NTNMCVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDGKVTQKEVKEFFRWAQDHVTEV						480
3	NTNMCVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDGKVTQKEVKDFFRWASDHVTDV						480
4	NTNMCVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDGKVTQKEVKDFFRWASDHVTDV						480
5	NTNMCVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDGKVTQKEVKDFFRWASDHVTEV						480
6	NTNMCVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDGKVTQKEVKDFFRWAKDHVVEV						480
7	NTNMCVVVDGNSTTFEHQQPLEDRMFKFELTRRLPDGKITQKEVKDFFAWAKVNQVPV						476
C	NTNMC*V*DGNSTTFEHQQPL*DRMFKFELT+RL:*DFGK*TKQEVK+FF*WA:****+V						

	490	500	510	520	
1	AHEFYVRKGGANKRPAPDDADKSEPKRA-----CPSVADPSTSDAEG				522
2	AHEFYVRKGGANKRPAPDDADKSEPKRA-----CPSVADPSTSDAEG				522
3	AHEFYVRKGGAKKRPASNDADVSEPKRQ-----CTSLAQPTTSDAEA				522
4	AHEFYVRKGGAKKRPASNDADVSEPKRQ-----CTSLAQPTTSDAEA				522
5	THEFYVRKGGARKRPAPNDADISEPKRA-----CPSVAQPSTSDAEA				522
6	EHEFYVKKGGAKKRPAPSDADISEPKRV-----RESVAQPSTSDAEA				522
7	THEFKVPRELAGTKGAEKSLKRPLGDTVNTSYKSLEKRARLSFVPETPRSSDVTVDPAPL				536
C	:HEF*V+***A:***A:****.*****: +:****:***A*:				

	530	540	550	560	570	580	
1	APVDFADRYQNKCSRHAGMLQMLFPCKTCERMNQNFNICFTHGTRDCSECFP--GVSESQ						580
2	APVDFADRYQNKCSRHAGMLQMLFPCKTCERMNQNFNICFTHGTRDCSECFP--GVSESQ						580
3	P-ADYADRYQNKCSRHVGMNLMFLPCKTCERMNQISNVCFTHGQRDCGECFPGMSSESQPV						581
4	P-ADYADRYQNKCSRHVGMNLMFLPCKTCERMNQISNVCFTHGQRDCGECFPGMSSESQPV						581
5	P-VDYADRYQNKCSRHVGMNLMFLPCRQCERMNQNVDFCFTHGVMDCAECFP--VSESQPV						580
6	S-INYADRYQNKCSRHVGMNLMFLPCRQCERMNQNSNICFTHGQKDCLECFP--VSESQPV						579
7	RPLNWNRSRYDCKCDYHAQFDNISNKCDECEYLNRGKNGCICHNVTHCQICHG-----						588
C	:::++**RY**KC**H:***:*****C::CE**N*::*:C**H*::*C.*C**...::+:::						

	590	600	610	620	
1	PVVRKRRTYRKLCAIHHLGRAPEIACSACDLVNVDLDDCVSEQ				623
2	PVVRKRRTYRKLCAIHHLGRAPEIACSACDLVNVDLDDCVSEQ				623
3	SVVKKKTYQKLCPIHHILGRAPEIACSACDLANVDLDDCVSEQ				624
4	SVVKKKTYQKLCPIHHILGRAPEIACSACDLANVDLDDCVSEQ				624
5	SVVRKRRTYQKLCPIHHIMGRAPEVACSACELANVDLDDCDMEQ				623
6	VSVVKKAYQKLCYIHHIMG-KVPDACTACDLVNVDLDDCIFEQ				621
7	-----IPPWEKENLSDFGDFDDANKEQ				610
C	:+*::+*::***:***:****+*::**D*DD*::EQ				

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